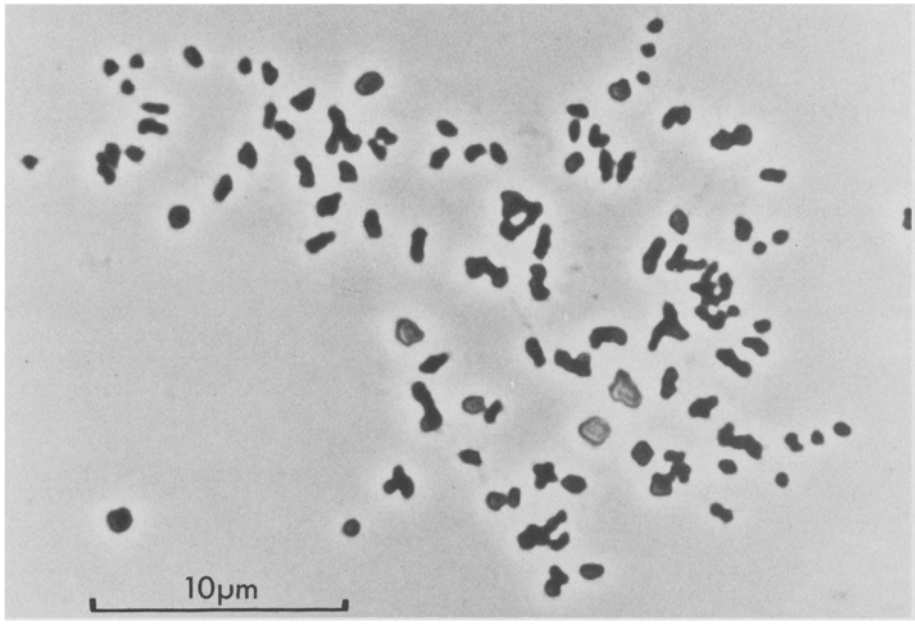

Fig. 2. Spermatocyte diakinesis with about 90 bivalents present.

tria. Such trends would be consistent with the modification of the *Mordacia* karyotype, including the reduction in chromosome number through such changes as centric fusions[9,13], and the possession of a large number of chromosomes in *Geotria.*

The information on the karyotype of *G. australis* presented in this paper suggests that this southern hemispheric species has the highest diploid number so far recorded for any vertebrate.

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Differential staining of a heterochromatic zone in *Arcyptera fusca* (Orthoptera)[1]

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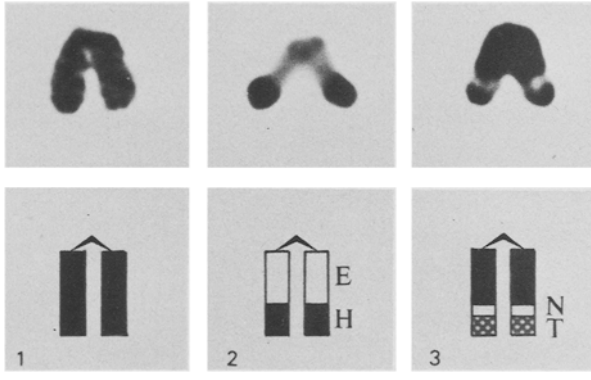
Summary. A homogeneous heterochromatic zone obtained with the direct application of the C-banding technique is unravelled into 2 sub-zones with the successive application of orcein staining and the C-banding method in the grasshopper *Arcyptera fusca.*

It has been argued that heterochromatin is the most dynamic of all chromosome components and although it has been intensively studied during the last years we still know too little about this component of the genome. The C-banding[2] and the N-banding techniques[3] are actually used as methods which offer a simple means of defining constitutively heterochromatic regions within the chromosome. However, there exist differences between the C and the heterochromatin N-banding patterns[4,5] within the same individual, which means that 2 different banding methods may reveal a specific differentiation within constitutive heterochromatin.

In this note, we report a singular case where a positive C-band, placed in the terminal region of a short autosome in the grasshopper *Arcyptera fusca* (Orthoptera: Acrididae), is unfolded with the combined action of both conventional orcein staining and the C-banding method. Testes of *A. fusca* are fixed in 3:1 ethanol: acetic acid for 24 h and stored in 70% ethanol. Squash preparations are made in lactopropionic orcein and frozen in liquid nitrogen. The coverslip is removed with a razor blade and left 1–2 days before proceeding further. Slides are steeped in a Coplin jar of freshly prepared 5% aqueous solution of barium hydroxide at 60 °C for 30 min, then rinsed thoroughly with

several changes of distilled water to remove scum that has formed. Preparations are incubated in 2×SSC (0.3 M sodium chloride containing 0.03 M trisodium citrate, adjusted to pH 7 with 0.1 citric acid) at 60 °C for 30 min, rinsed in distilled water and stained in buffered Giemsa (G.T. Gurr) 3% solution for 3–5 min. Finally they are rinsed briefly in deionised water and dried in air. Mount in 'De Pex' or a similar mountant.

A. fusca presents a male chromosome complement 2n=22+X. The autosomes (L_1-L_2, M_3-M_8, S_9-S_{11}) and the X chromosome are acrocentric. The pattern of C-banding shows 2 principal categories defined by their localization within the karyotype: paracentromeric, in the vicinity of the centromere of all the chromosomes and distal in M_4 to S_{11} chromosomes (Gonsálvez et al., in preparation). S_{10} autosome particularly shows 2 big distal blocks of constitutive heterochromatin clearly visible when the C-banding procedure is used (figure, 2). This pattern of pycnosis differs from that obtained just with orcein stain (figure, 1)



S_{10} selected univalents and diagrams of *Arcyptera fusca;* metaphase II. *1* A selected univalent with orcein staining. *2* Same univalent with C-banding. *3* Same univalent with orcein staining and C-banding. E, euchromatin; H, heterochromatin; N, non-staining gap; T, terminal heterochromatin.

or when an orcein stain is applied before the C-banding technique. This latter method gives rise to a banding pattern which shows 2 bands within the zone occupied by the constitutive heterochromatin obtained with the C-banding procedure (figure, 3). We have studied about 150 males from different populations and all of them were homozygous for the 2 big heterochromatic blocks in chromosome S_{10}. 20 of them were processed to unravel the band and we obtained good results in 12 of them. Mitotic chromosomes in sufficient numbers for a comparison have not been available so far.

It is interesting to note the similarity between the non-staining gaps (figure, N in 3) and those obtained in the grasshopper *Warramaba virgo* with the G-banding method[6]. But while in *W. virgo* all these gaps correspond well with the bands that were darkly stained by the C-banding technique, in *A. fusca* just half of the C-positive band shows a non-staining gap which means that the supposed homogeneous heterochromatic band (figure, H in 2) has been unfolded. 2 different bands can be observed within this region; one of them is a telomeric positively stained (T) and the other a negatively stained band (N). One might speculate that saturated barium hydroxide causes a different effect in the DNA when the orcein is present in the structure of the chromosome, but a role for base composition in the resistance of heterochromatin bands has not yet been ruled out[7].

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Human chromosomal heteromorphism in American blacks. V. Racial differences in size variation of the short arm of acrocentric chromosomes[1]

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Summary. Normal American blacks were studied by the RFA (R-bands by fluorescence using acridine orange) technique to estimate the frequency of size variation of the short arm of acrocentric chromosomes and to compare the data on Caucasians.

Heteromorphisms of human chromosomes have been recognized since the early 1960s. Racial differences in the size and morphology of the short arms of human chromosomes in D and G groups have been recognized without banding techniques[2]. Improved banding techniques have permitted recognition of greater numbers of chromosomal heteromorphisms[3]. To our knowledge, there is only one study where the size of D and G group chromosomes have been examined in American blacks and Caucasians using conventional staining. Individual chromosomes of D (13, 14 and 15) and G (21 and 21) groups could not be indentified in their study. Consequently, they could not demonstrate racial differences for each acrocentric chromosome but reported by group (D and G). Recently, we developed a RFA technique (R-bands by fluorescence using acridine orange as suggested by the Paris conference[4–6]) which distinguishes each human chromosome with absolute certainty[7]. One of the most important advantages of this banding technique is that the short arms of human acrocentric chromosomes are well delineated and minor differences can easily be detected. Consequently, we utilized the RFA technique to demonstrate racial differences in the size of human acrocentric chromosomes in 100 American blacks and 100 Caucasians. To our knowledge, this is the first